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Morphological and molecular identification of *Geophagus sveni* Lucinda, Lucena & Assis, 2010 (Cichlidae, Cichliformes) from the Paraná river basin, Argentina

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Abstract

During 2015, we collected several specimens of a cichlid tentatively assigned to *Geophagus* in Yacyretá reservoir in the Paraná river basin (Argentina). By means of morphological and molecular evidence, we identified these specimens as *Gephagus sveni*, a species known from middle portion of the Tocantins River. Here we report the presence of the genus *Geophagus* (sensu stricto) in Argentina for the first time.

Key words

New record; freshwater fish; Argentine ichthyofauna; cytochrome oxidase 1; acara.

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Introduction

The family Cichlidae is a very diverse fish group with 1711 species (Fricke et al. 2018) distributed in fresh- and brackish waters of North, Central, and South America, Africa, the Jordan Valley in the Middle East, Madagascar, Iran, southern India, and Sri Lanka (Kullander 2003). Historically classified as part of the taxonomically conflictive Perciformes, recent phylogenetic works suggested that together with monotypic Pholidichthyidae, they belong to the order Cichliformes (Betancur-R. et al. 2013, Mirande 2017, Betancur-R. et al. 2017, Ilves et al. 2018).

The genus *Geophagus* Heckel, 1840 are Neotropical cichlids that belong to the subfamily Cichlinae. This genus was originally diagnosed to include large cichlids with an expanded anteroventral lamina on the first epibranchial, lined with gill-rakers. Based on the number of supraneural bones, Gosse (1976) divided the genus into *Gymnogeophagus* Miranda Ribeiro, 1918 with 2 supraneurals, *Geophagus* with 1, and *Biotodoma* Eigenmann & Kennedi, 1903 without a supraneural bone. Later on Kullander (1986) resurrected *Satanoperca* Günther, 1862 and redefined *Geophagus* to include only species with a swimbladder prolongation into the caudal region, which is lined by 6–12 epihemal ribs and also more caudal than precaudal vertebrae. Those characters define the *Geophagus* sensu stricto species group and are absent in the species from the "*Geophagus*" *brasiliensis* and "*Geophagus*" *steindachneri* species groups. Molecular phylogenetic studies (López-Fernández et al. 2010, Ilves et al. 2018) also split species of *Geophagus* in these 3 different clades. *Geopha-*

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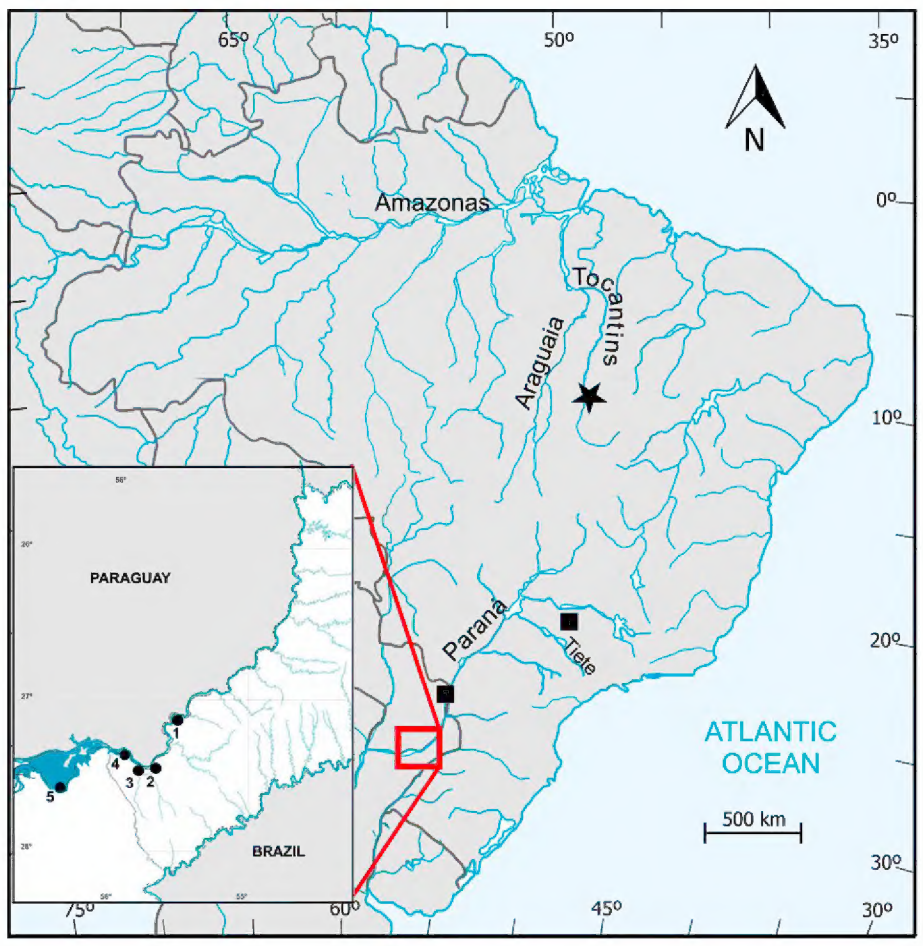

Figure 1. Distribution of *Geophagus sveni*. ★ = type locality of *G. sveni*. ■ = specimens identified at GenBank as *G. proximus* but whose genetic distance with *G. sveni* defined herein is very low (D ≤ 0.2%). ● = localities reported in this work: 1 = Puerto Maní, 2 = Candelaria, 3 = Garupá, 4 = Posadas, 5 = Santa Tecla (all in Argentina).

gus sensu stricto and "*Geophagus*" *steindachneri* groups are related to *Gymnogeophagus* (Geophagines), whereas "*Geophagus*" *brasiliensis* group is more closely related to *Mikrogeophagus* (Mikrogeophagines) and *Biotodoma* (Ilves et al. 2018).

Geophagus sensu stricto is currently composed of 20 valid species widely distributed in South America and adjacent Central America (Deprá et al. 2014). Although the Amazon native *Geophagus proximus* (Castelnau, 1855) has been recorded from reservoirs of the Upper Paraná river basin in Brazil since the early 2000s (Graça and Pavanelli 2007, Moretto et al. 2008, Gois et al. 2015), there were no records of the genus from the Argentine stretch until now (Mirande and Koerber 2015). In a recently published update to Graça and Pavanelli (2007), Ota et al. (2018) determined as *Geophagus sveni* Lucinda, Lucena & Assis, 2010 specimens previously assigned to *Geophagus* cf. *proximus*. In this paper, we corroborate the presence of *G. sveni* in Argentina on the basis of morphological analyses and corrected pairwise genetic distances of a fragment of mitochondrial gene Cytochrome oxidase subunit I (*COI*); and also discuss the presence of *G. proximus* in the Paraná river basin.

Methods

Specimens were collected by means of gillnets at different upstream points of the Yacyretá Reservoir in the Paraná River, Corrientes and Misiones provinces, Argentina (Fig. 1). Fishes were euthanized by overdose in benzocaine anesthetic solution (Close et al. 1996, Neiffer and Stamper 2009), fixed in a 10% formalin solution, preserved in 70% ethylic alcohol, and deposited at the "Colección Ictiológica, Laboratorio de Génetica Evolutiva, Universidad Nacional de Misiones" (LGEP). Measurements and counts were performed in 11 specimens (Table 1) following Kullander and Nijssen (1989) and Kullander et al. (1992). Specimen LGEP367 was cleared and stained according to Taylor and Van Dyke (1985) for osteological analysis.

DNA sequence analysis. Muscle tissue samples for molecular studies were obtained post-mortem and preserved in 100% ethylic alcohol. Total genomic DNA was extracted from ethanol-preserved muscle tissue of specimen LGEP452, using the Qiagen DNeasy kit. PCR amplifications were carried out in 30 µl reactions using 0.2 µl Taq (Genbiotech). A 650-bp DNA sequence from the 5′ region of mitochondrial gene Cytochrome oxidase subunit I (COI), was amplified using the cocktail primers: VF2_t1; FishF2_t1; FishR2_t1; Fr1d_t1 (Ivanova et al. 2007). The PCR protocol consisted of an initial denaturation step at 95 °C (2 min), 30 cycles consisting of 94 °C (30 s) for denaturation, 54 °C (30 s) for annealing, and 72 °C (1 min) for extension followed by a final extension step at 72 °C (10 min) (Ward et al. 2005). PCR-amplified products were cleaned using AccuPrep PCR Purification Kit. The products were sequenced with an automated sequencer (Macrogen, Korea) and all samples were sequenced in both directions to check for potential errors. Chromatograms obtained from the automated sequencer were processed and edited using ChromasPro Version 2.1.2 (Technelysium Pty Ltd) and deposited in

Table 1. Morphological measurements and counts of *Geophagus sveni* from Paraná River in Misiones, Argentina.

Measurements	*n*	Range	Mean	SD
SL (mm)	11	96.1–163.25	*	*
Percents of SL				
Head length	11	29.02–31.16	30.31	0.64
Body depth	11	42.72–48.48	45.53	2.11
Body depth sin dorsal	11	39.26–46.62	42.75	1.98
Caudal peduncle depth	11	11.84–14.61	13.48	0.90
Caudal peduncle length	11	15.8–22.95	21.23	2.03
Pectoral fin length	11	36.03–43.42	39.80	2.34
Pelvic fin length	11	39.43–68.09	52.98	9.40
Last D spine length	8	17.4–19.42	18.37	0.68
Percents of HL				
Snout length	11	48.78–59.41	54.14	3.01
Orbital diameter	11	21.91–27.59	25.06	1.77
Head depth	11	110.89–136.50	119.60	6.55
Head width	11	46.81–56.41	52.55	2.73
Interorbital width	11	25.59–32.76	28.55	2.01
preorbital depth	11	37.15–46.66	42.37	3.03
Counts	***n***	**Range**	**Modal value**	**SD**
E1 scales	9	32–35	34	0.95
H scales	10	6–7	7	0.32
ULL scales	10	21–23	21.22	0.67
LLL scales	11	15–18	18	1.08
Dorsal fin rays	10	(XVII–XVIII)+(11–12)	XVII+12	0.60
Pectoral fin rays	11	14–15	15	0.40
Pelvic fin rays	11	I+5	I+5	0
Anal fin rays	10	III+7–III+8	III+8	0.32

GenBank under the accession numbers MH780911. For comparison, *COI* sequence of a *Geophagus sveni* from Tocantins river basin was used (voucher material LBP-17378 is stored at Laboratório de Biologia e Genética de Peixes, Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Botucatu. São Paulo). Sequences from various species of *Geophagus*, available at GenBank (http://www.ncbi.nlm.nih.gov/Genbank) and BOLD (http://www.barcodinglife.org) were also used. Employed sequences and references are shown in Table 1. Pairwise genetic distances were calculated for fragments of 543 pb using MEGA, version 6 (Tamura et al. 2013) to estimate genetic divergence (K2P distances) between our specimen, *G. sveni* from Tocantins and other species of *Geophagus* (Table 2).

Results

New records. All specimens collected by Aichino DR, Cerutti JC and Massin SA. Argentina. Misiones, Garupá, Garupá stream (27°29′34″ S, 055°49′07″ W): October 30, 2015 (7 specimens, LGEP 364); December 17, 2015 (2 specimens, LGEP 366); February 12, 2015 (1 specimen, LGEP 367); March 19, 2015 (1 specimen, LGEP 395); April 15, 2015 (1 specimen, LGEP 396); April 15, 2015 (1 specimen, LGEP 397); February 12, 2015 (1 specimen, LGEP 400). From Corrientes, Santa Tecla, Paraná River (27°26′17″ S, 056°22′31″ W): February 17, 2015 (1 specimen, LGEP 394). Misiones, Corpus, Paraná River (27°06′21″ S, 055°30′51″ W): May 20, 2015 (3 specimens, LGEP 398). Misiones, Candelaria, Paraná River (27°26′53″ S, 055°43′53″ W): June 12, 2010 (1 specimen, LGEP 399). Misiones, Posadas, Paraná River (27°21′16.4″ S, 055°54′14.0″ W) June 28, 2016 (1 specimen, LGEP 452) collected by Torres J.

Identification. The presence of paired caudal extensions of the swimbladder lined by 6–12 epihemal ribs and more caudal than precaudal vertebrae allowed us to assign the studied specimens to genus *Geophagus* sensu stricto. Correspondence of specimens to the nominal species *Geophagus sveni* (Fig. 2) was based on the absence of a suborbital stripe, the lack of a preopercular mark and the possession of 5 faint, vertical, parallel, solid bars on the flank.

The absence of a head stripe distinguish *G. sveni* from all the species outside the *G. surinamensis* complex [*G. brasiliensis* (Quoy & Gaimard, 1824); *G. obscurus* (Castelnau, 1855); *G. crassilabris* Steindachner, 1876; *G. iporangensis* Haseman, 1911; *G. itapicuruensis* Haseman, 1911; *G. pellegrini* Regan, 1912; *G. steindachneri* Eigenmann, 1922; *G. harreri* Goose, 1976; *G. argyrostictus* Kullander, 1991; *G. grammepareius* Kullander & Taphorn, 1992; *G. taeniopareius* Kullander & Royero, 1992; *G. gottwaldi* Schindler & Staeck, 2006; *G. diamantinensis* Mattos, Costa & Santos, 2015; *G. rufomarginatus* Mattos & Costa, 2018; *G. multiocellatus* Mattos & Costa, 2018 and *G. santosi* Mattos & Costa, 2018], the absence of a dark preopercular mark noticeable in live and alcohol-preserved specimens distinguish it from *G. proximus*; *G. brachybranchus* Kullander & Nijssen, 1989; *G. dicrozoster* López-Fernández & Taphorn, 2004; *G. winemilleri* López-Fernández & Taphorn, 2004 and *G. crocatus* Hauser & López-Fernández, 2013. *Geophagus*

Table 2. List of *COI* sequences employed for genetic distance estimation.

Species	GenBank or BOLD Id	Specimen catalogue	GenSeq nomenclature	Latitude	Longitude
G. sveni	MH78911	LGEP452	genseq-4	27°21′16.4″ S	055°54′14.0″ W
G. sveni	MK12088	LBP-17378	genseq-4	10°07′59.6″ S	048°18′53.0″ W
G. proximus	GU701783	LBP-37221	genseq-4	21°14′44.2″ S	048°17′50.3″W
G. proximus	GU701784	LBP-37220	genseq-4	25°25′11.3″ S	054°32′08.2″W
G. proximus	GU701785	LBP-37223	genseq-4	21°14′44.2″ S	048°17′50.3″W
G. proximus	GU701786	LBP-37222	genseq-4	21°14′44.2″ S	048°17′50.3″W
G. proximus	JN988869	LBPV-37219	genseq-4	25°25′11.3″ S	054°32′08.2″W
G. dicrozoster	DSFRE170-08	Not provided	*	*	*
G. dicrozoster	DSFRE171-08	Not provided	*	*	*
G. surinamensis	JN026710	GESU-Petshop-1	genseq-4	*	*
G. surinamensis	KU568829	ES12-AT028	genseq-4	*	*
G. argyrostictus	PARO178-08	Not provided	*	*	*
G. argyrostictus	PARO177-08	Not provided	*	*	*
G. harreri	DSFRE369-08	Not provided	*	*	*
G. steindachneri	UDEA115-18	CIUA-8855	genseq-4	5°30′03.9″ N	074°41′13.9″W
G. steindachneri	UDEA116-18	CIUA-8868	genseq-4	5°30′03.9″ N	074°41′13.9″W
G. pellegrini	MG936927	stri-6733	genseq-4	8°37′33.2″ N	077°49′01.2″W
G. pellegrini	MG936928	stri-1764	genseq-4	8°50′45.2″N	077°41′17.5″W
G. crassilabris	MG936924	stri-12254	genseq-4	9°16′25.7″ N	078°40′52.7″W
G. crassilabris	MG936925	stri-3618	genseq-4	8°58′45.8″ N	078°30′20.2″W
G. brasiliensis	JN988864	LBPV-40176	genseq-4	22°22′42.2″ S	047°12′37.8″W
G. brasiliensis	KP218743	CT2506	genseq-4	19°30′00.0″ S	042°22′48.0″W
G. proximus	HM064993	LBP-16081	genseq-4	22°00′00.0″ S	041°19′58.8″W
G. proximus	HM064991	LBP-16084	genseq-4	22°00′00.0″ S	041°19′58.8″W

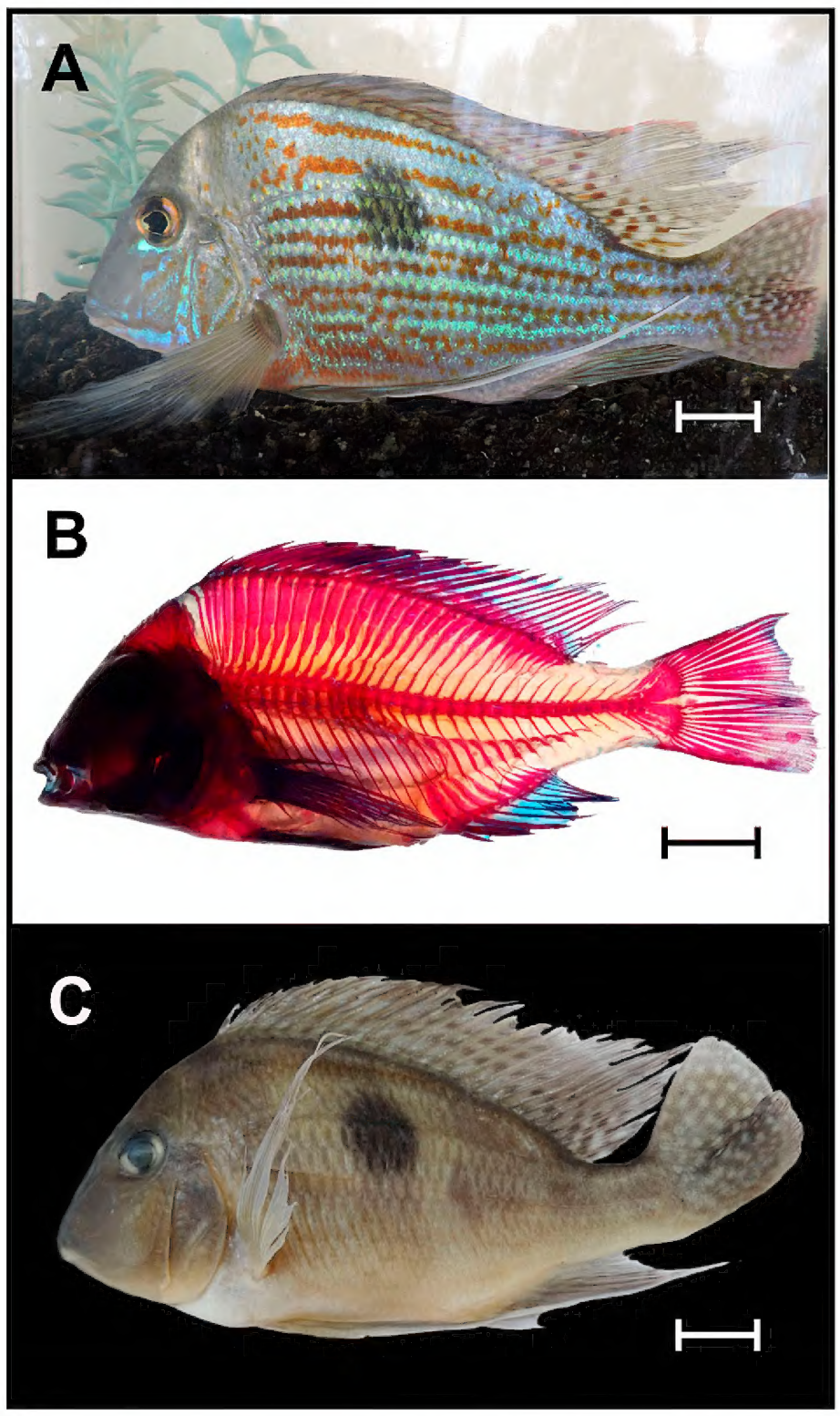

Figure 2. *Geophagus sveni*. **A.** Live coloration. **B.** Cleared and stained specimen. **C.** Ethanol-preserved specimen. **A** and **C** correspond to voucher LGEP398 (161.7 mm) and **B** to LGEP367 (130.7 mm). Scale bars = 20 mm.

sveni is distinguished from *G. megasema* Heckel, 1840; *G. camopiensis* Pellegrin, 1903; *G. altifrons* Heckel, 1840; *G. surinamensis* Bloch, 1791; *G. abalios* López-Fernández & Taphorn, 2004; *G. brokopondo* Kullander & Nijssen, 1989; *G. neambi* Lucinda, Lucena & Assis, 2010 and *G. mirabilis* Deprá, Kullander, Pavanelli & da Graça by having 5 faint, vertical, parallel, solid bars on the body flank and absence of head marks. Only *G. parnaibae* Staeck & Schindler, 2006 has 5 bars but second and third are medially bisected unlike that of *G. sveni*, which are solid. Also caudal fin color pattern distinguished *G. sveni* (alternating vertical white and dark bars) from *G. parnaibae* (alternating horizontal white and dark bars).

Molecular identification by means of mitochondrial gene *COI* supports the phenotypical determination. The pairwise analysis of *COI* sequence distances, revealed no intraspecific variation among specimen LGEP 452 from Paraná River and topotypic specimen LBP–17378. Besides, scarce (≤ 0.2%) or no differences were found between these, and the sequences GU701783, GU701785, GU701786, and JN988869 stored in GenBank as *G. proximus*. The remaining sequences from GenBank identified as belonging to *G. proximus* were found to be more related to '*G*'. *brasiliensis* species group (D ≥ 15%). Thus, they probably do not represent specimens of *Geophagus sensu stricto*.

Discussion

The finding of *Geophagus sveni* in the Paraná River in Argentina constitutes the first report of the genus *Geophagus* sensu stricto from the country; although the presence of the genus in the Paraná river basin has been suggested since 2007, with reports of *G. proximus* (Graça and Pavanelli 2007, Moretto et al. 2008, Gois et al. 2015). The first report of *G. sveni* in the Upper Paraná river basin is an updated checklist of fishes from the Upper Paraná floodplain by Ota et al. (2018). These authors reassigned specimens previously reported as *Geophagus* cf. *proximus* (Graça and Pavanelli 2007) to *G. sveni*. Additionally, *G. proximus* was reported from 3 reservoirs of middle and lower Tietê (Moretto et al. 2008) and Upper Paraná River (Gois et al. 2015). Those authors found a correlation between the population growth of *G. proximus* and decreased abundance of *Satanoperca pappaterra* Heckel, 1840. We were not able to estimate if there is an impact on fish assemblage caused by *G. sveni*, but Graça and Pavanelli (2007) suggested that the species establishment was recent but successful.

Analysis of genetic distances revealed that the specimen here analyzed (LGEP452) has no difference with the specimen LBP–17378 from Tocantins River; as well as scarce or no differences were found between these and *COI* sequences stored in GenBank as *Geophagus proximus* (Table 3). These *G. proximus* sequences belong to specimens captured in the Upper Paraná river basin, but considering the usual problems of GenBank with misidentifications, we feel it pertinent that a careful revision of voucher material be made to accurately determine which species those sequences belong to. That will help establish a better understanding of the distribution of *G. sveni* and *G. proximus*. The correct determination of these cichlid species and the monitoring of fish assemblages are necessary to determine if we are seeing an invasive species expanding southwards. If so, measures can be taken aimed at containing or controlling its spread.

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Table 3. *COI* gen K2P distance matrix of *Geophagus*.

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